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DECLARATION OF DR. WALTER KING PURSUANT TO 37 CFR 1.132

I, Dr. Walter King, declare as follows:

1. I am presently the Vice President of Research & Development at GE Lifesciences, part of GE Healthcare USA, and I am based in the facility in Sanford, Maine. I have been employed in this position since 2006. I am an experienced Ph.D. with an extensive background in Bacteriology & Immunology, Virology, and Microbiology & Urology. I am responsible for managing all aspects of global research and development activities at Whatman PLC, including development and implementation of the product portfolio. Prior to my current position at Whatman, I had previously (2005-2006) been employed as a Director of the Applications Development Group at Nanosphere, Inc., in Northbrook, IL, where I was in charge of development of In Vitro Diagnostic (IVD) products for genetic and infectious disease testing using single nucleotide polymorphism detection technology in patient samples without amplification. Prior to that, I had been a Senior Director of Product Development in the Vysis Diagnostic Division and Molecular Oncology Program Director at Abbott Laboratories (2002-2005) in Downers Grove, IL, where I was in charge of development of

microarray IVD products for molecular cytogenetics and oncology; and a Director of the Genomic Microarray Platform program (1999-2001; genomic array-based assay products for the detection of amplification and deletions for solid tumors, leukemias, lymphomas and prenatal applications) and a Senior Manager of Assay Development (1995-1998; IVD's for Her-2 detection in breast cancer [PMA approval], multiplex aneuploidy detection in bladder cancer [510K approval], and pre-implantation testing in blastomeres and polar bodies) at Vysis, Inc. in Downers Grove, IL. I was also a Senior Scientist in Assay Development (1986-1995; assay formats and chemistries for an automated clinical analyzer; amplification chemistry for detection of respiratory, gastrointestinal and sexually transmitted disease panels; manual non-isotopic probase-based test for detection of Listeria, Salmonella and E. coli) at Gene-Trak Systems, Inc., in Framingham, MA; and was employed in the Biotechnology Group (1985-1986; development of nucleic acid probe technology for the detection of clinical pathogens) of AMOCO Corporation in Naperville, IL. I have been active in the development of various products, have been listed as an inventor on several patents, and have co-authored over thirty publications. I hold a Bachelor of Arts (1973) from the Department of Bacteriology and Immunology of the University of California in Berkeley, CA. I hold a Ph.D. in Virology (1980) from the University of Chicago in Chicago, IL. I served as a Postdoctoral Fellow in the Department of Medicine at the University of Chicago (1980-1982), where I identified the transforming region in the Epstein-Barr Virus (EBV) genome, and in the Departments of Microbiology and Urology at Columbia University Medical Center in New York, NY (1982-1985), where I identified genes involved in the differentiation of embryonal carcinoma cells. I am a member of the American Association for Cancer Research.

2. The subject application discloses among other things and claims a method of isolating or purifying one or more plasmids from a host cell or virus comprising: a) providing a dry matrix or solid medium, wherein said dry matrix or solid medium further comprises: i) a weak base; ii) a chelating agent; and iii) an anionic surfactant or an anionic detergent; b) contacting the matrix or solid medium with a sample comprising a host cell or virus containing said plasmid or plasmids; and c) isolating all or a portion of said plasmid or plasmids from said matrix or solid medium. It also discloses among other things and claims a

method of isolating or purifying one or more plasmids from a host cell or virus comprising: a) contacting a solid medium with a sample comprising a host cell or virus containing said plasmid, wherein the solid medium comprises: i) a polymeric matrix comprising a cellulose-based matrix, a micromesh synthetic polymer matrix, or a micromesh synthetic plastic matrix; ii) a weak base; iii) a chelating agent; and iv) an anionic surfactant or an anionic detergent; b) releasing the plasmid from the host cell or virus and onto said medium; and c) isolating said plasmid from said medium. It also discloses among other things and claims a method of isolating or purifying one or more plasmids from a host cell or virus comprising: a) contacting a solid medium with a sample comprising a host cell containing said plasmid, wherein said solid medium protects against degradation of said plasmid and wherein the solid medium comprises: i) a polymeric matrix comprising a cellulose-based matrix, a micromesh synthetic polymer matrix, or a micromesh synthetic plastic matrix; and ii) a composition sorbed to the polymeric matrix, wherein the composition comprises: a weak base; a chelating agent; and an anionic surfactant or an anionic detergent; b) lysing the host cell; c) releasing the plasmid from the host cell and onto said medium; and d) isolating said plasmid from said medium.

3. For many projects, generation of numerous DNA samples from biological specimens is routine. Traditionally, however, handling and archiving a large collection can become a logistical problem for the laboratory. Previously, there had often been a need to use organic solvents or harsh chemicals or to store samples in a freezer. One solution, used in forensic labs, was the bloodstorage medium FTA® Cards. An FTA® Card could be used ***to store genomic*** DNA in the form of dried spots of human whole blood, the cells of which were lysed on the paper. Before analysis of the captured ***genomic*** DNA, a few washing steps would be used to remove the stabilizing chemicals and cellular inhibitors of enzymatic reactions, but the ***genomic*** DNA would largely remain on the card. ***Isolated bacterial*** DNAs ***spotted*** on FTA® Cards could be an efficient system for storage and retrieval as well, followed by downstream applications, such as PCR ribotyping. Further, ***purified*** plasmid DNA was spotted on treated paper and recovered. Nevertheless, there was a need for a simplified

method of ***isolation and storage*** of ***plasmid*** DNA ***directly from cells*** onto a dry matrix or solid medium ***without prior isolation of the plasmid DNA from the host***, followed by its ***subsequent isolation*** from the matrix or solid medium.

4. The current invention addresses these concerns and many other issues as well, such as protection from degradation.

5. I have reviewed the Patent Office Action ("Office Action") dated September 26, 2008, issued in connection with the subject application. As I understand the Office Action, the Patent Examiner has rejected certain claims of the application in view of documents that include the following: Fujishiro (JP 07-250681; 10 March 1995; translation provided by JPO@http://www.ipdl.inpit.go.jp/; "Fujishiro"); Rogers et al. (Anal. Biochem., 247(2): 223-227; May 1, 1997; "Rogers & Burgoyne" or "Rogers"); U.S. Patent 5,496,562 (Burgoyne; granted March 5, 1996; "Burgoyne"); and Kahn et al. (Methods Enzymol., 68: 268-280; 1979; "Kahn").

6. I disagree with these claim rejections.

7. The current invention illustrates that it is possible provide **methods** to **isolate or purify** a **plasmid** from a host cell on a **dry matrix or solid medium** comprising **a weak base, a chelating agent, and an anionic surfactant or an anionic detergent by contacting the matrix or solid medium with a sample** comprising a host cell containing the **plasmid**, and **subsequently isolating the plasmid from the matrix or solid medium**. The method may also comprise **lysis of the host cell upon contact with the solid medium to release the plasmid** from the host cell onto the medium. The **solid medium also protects against degradation** of the plasmid. In **some** embodiments, the **solid medium comprises**: i) a **polymeric matrix** comprising a **cellulose**-based matrix, a **micromesh synthetic polymer** matrix, or a **micromesh synthetic plastic** matrix; **and** ii) a **composition sorbed to the**

polymeric matrix, the composition comprising **a weak base, a chelating agent, and an anionic surfactant or an anionic detergent**.

8. In contrast, Fujishiro neither discloses nor suggests the present invention.

9. Fujishiro **does not disclose a dry matrix or solid medium** comprising **a weak base, a chelating agent, and an anionic surfactant or an anionic detergent,** whereby the **plasmid is isolated from the host cell onto the matrix or solid medium.** Rather, **Fujishiro is directed to a sequential method of plasmid purification** that not only calls for the **removal of RNA** (see, e.g., [0011]), but also for **sequential movement (and purification of the plasmid DNA) into a second cartridge,** this one **containing glass in fiber or powder form** (see, e.g., [0020]-[0025]). Fujishiro **neither describes nor suggests sorbing the lysing agents to yield a dry matrix or solid medium.** Moreover, Fujishiro **teaches away from such a matrix or medium** by the fact that Fujishiro identifies sequential processing steps of lysis, digestion of RNA, and transport to a DNA binding medium, which can only mean that Fujishiro **never envisioned that all steps could be embodied on a single surface with less processing.** Instead, Fujishiro **emphasizes the need for sequential use of separate cartridges.**

10. In the "Means for Solving the Problem" (see [0005]), **Fujishiro describes a process having a *first* cartridge for bacteriolysis and a decomposition process for RNA, *followed by* adsorption of DNA, washes, and elution in a *second* cartridge.**

11. For instance, the user first adds a liquid culture to the first cartridge (see [0009]), and it is only then that the user adds other chemicals for bacteriolysis and RNA degradation (see [0010]-[0012]). The specification describes the addition of lysozyme (bacterial cell wall hydrolase) for bacteriolysis and RNase A (ribonuclease A) for decomposition of RNA (see [0011]). After treatment for bacteriolysis and decomposition of RNA, the user performs "full solubilization processing" of a sodium lauryl sulfate/0.2 N sodium hydroxide solution for 2-5 minutes, followed by the addition of 3M potassium acetate

(pH 4.8) for 3-5 minutes, then coagulation of cell structural proteins and chromosomal DNA by filtration actuation, either by using a vacuum pump or by centrifugation of the first cartridge so that the plasmid DNA is in the lower part of the first cartridge (see [0012]). The plasmid DNA-containing fraction is then transferred to the second cartridge, where sodium iodide (e.g., 8M) is added (see [0013]) is added. The second cartridge consists of at least a four-fold structure of a glass fiber filter (bilayer), a glass powder layer, and a membrane filter. Actuation using reduced pressure via a vacuum pump or using centrifugation is employed to cause the plasmid DNA to adsorb ("stick") to the second cartridge (see [0013]). **Fujishiro states that "<u>[p]lasmid DNA</u> is mainly adsorbed by the <u>glass powder</u> layer"** (see [0013], all emphasis added). After adsorption, the user washes the DNA with ethanol and ethylenediaminetetra-acetic acid (1mM EDTA, pH 8.0) with actuation, either by using a vacuum pump or by centrifugation, followed by elution of the plasmid DNA in sterile distilled water/10mM Tris-hydrochloric acid (pH 8.0) and sterile distilled water/1mM Tris-hydrochloric acid (pH 8.0), and 50-100 "microl" EDTA (see [0013]).

12. In another example, Fujishiro describes the first cartridge, which is shown in Drawing 1 (see [0014]-[0019] and Drawing 1). **The Office Action refers specifically to the filter compositions of this portion of the specification.** However, **the <u>trap filter</u>** (1), which may be a glass fiber filter, a polyethylene resin filter, a non-woven fabric filter, or other material, **is the layer used for <u>uptake of the "fungus bodies"</u> resulting from <u>bacteriolysis</u> – <u>not</u> <u>for the isolation of the plasmid DNA</u>** (see [0016], all emphasis added). Moreover, **the <u>membrane filter</u>** (2), which may be cellulose acetate, polyvinylidene fluoride, or other material, is **"mainly a layer <u>for filtration and removal of discard</u>, such as <u>coagulation protein and Chromosome DNA</u>"** – again not for the isolation of the plasmid DNA (see [0017], all emphasis added). Rather, **<u>the isolation of plasmid DNA takes place in the second cartridge</u>**, which is shown in Drawing 2 (see [0020]-0025] and Drawing 2). The **composition and function of the membrane filter (21) in the second cartridge** are **the same as the composition and function of the membrane filter (2) in the first cartridge,** namely, "mainly a layer **<u>for filtration and removal of discard</u>, such as <u>coagulation protein and Chromosome DNA</u>" – <u>not</u> <u>for the isolation of the plasmid DNA</u>** (see [0017] and

[0023], all emphasis added). Instead, "**[t]he glass powder layer 22 is mainly a layer for DNA adsorption**" (see [0022], all emphasis added). This layer is prepared from a glass powder suspension and is sandwiched between **two glass fiber filters** (21, 23), which "**are mainly the layers for plasmid adsorption assistance**" (see [0020]-[0022], [0024] and Drawing 2, all emphasis added). ***In essence, the sample encounters a series of filters in the first and second cartridges with the isolation of the plasmid DNA taking place on the glass powder layer in the second cartridge.***

13. In yet another example, a trial was conducted using the first cartridge (Drawing 1) and the second cartridge (Drawing 2) (see [0027]-[0032]). ***Bacteriolysis and impurity filtration*** were performed **in the *first* cartridge, *followed by plasmid DNA adsorption on the glass fiber filter and the glass powder of the second cartridge*** (see [0029]-[0031]). The plasmid DNA was eluted from the glass and was subjected to agarose gel electrophoresis (see [0031]-[0032] and Drawing 3).

14. Again, Fujishiro **neither describes nor suggests sorbing the lysing agents to yield a dry matrix or solid medium.** Moreover, **Fujishiro does not disclose the present invention and even teaches away from such a matrix or medium** by the fact that Fujishiro identifies sequential processing steps of lysis, digestion of RNA, and transport to a DNA binding medium, which can only mean that Fujishiro **never envisioned that all steps could be embodied on a single surface with less processing.** Instead, Fujishiro **emphasizes the need for sequential use of separate cartridges.** Therefore, the disclosure of **Fujishiro does not disclose the present invention and, in fact, teaches away from the present invention**.

15. In addition, Rogers neither discloses nor suggests the present invention.

16. Instead, Rogers shows recovery of DNA from bacterial liquid cultures by application of the culture to the FTA® solid medium, followed by polymerase chain reaction (PCR) amplification of **genomic** DNA ***in situ* on FTA® medium**. Rogers tests only **bacterial strains**. The sequence that Rogers amplifies, and hence detects, is **not** **a vector** component

(and hence **not a plasmid**), but rather the ribosomal RNA genes of the **chromosomal/genomic DNA**. That this is the case can be seen from the Rogers & Burgoyne paper, page 224, column 2, paragraph headed "PCR Primers". Rogers does not show use of bacteria comprising vectors or media comprising micromesh plastic, and Rogers does not detail the composition of the chemicals in the FTA® medium, but references the Burgoyne patent. Moreover, there is no suggestion in Rogers that the cells in the samples contain both genomic DNA and plasmid DNA mixed together. Rogers does **not** demonstrate **plasmid** DNA isolation on a solid medium or matrix **directly from cells containing a mixture of both genomic DNA and plasmid DNA**.

17. Although the Rogers paper references the Burgoyne patent, the Rogers paper and the Burgoyne patent, taken either alone or together, neither disclose nor suggest the present invention.

18. The deficiencies of Rogers are **not** remedied by the disclosure of Burgoyne. Burgoyne describes a solid matrix having a compound or composition comprising uric acid, together with a weak base. Burgoyne alternatively describes a composition comprising a monovalent weak base (such as Tris), a chelating agent (such as EDTA), an anionic detergent (such as SDS), and optionally uric acid or a urate salt sorbed to a cellulose-based paper. However in Burgoyne, ***previously purified*** plasmid DNA is applied to the FTA® cards, and then the card is coated with a plastic polymer (polystyrene) to keep the card dry and/or preserve the DNA when stored in the freezer (Example 2). Burgoyne describes the application of **previously purified** plasmid pUC19 (i.e., purified from cellular components **and purified from genomic DNA**), whereby the **previously purified** plasmid pUC19 in solution in TE buffer is dried onto Whatman No. 1 paper, which has been soaked with a solution of 40 ml mM uric acid and 100 mM Tris (free base), and then is then sheathed in protective polystyrene and later chloroform extracted to remove the protective layers (Example 2.5; col. 5, l. 54, to col. 6, l. 21). In contrast to the present invention, **Burgoyne does not disclose plasmid vector DNA** isolated **directly from cells**, however. Rather, in Burgoyne, the plasmid DNA is first purified by state of the art methods at the time then

applied to the treated matrix. It is not intuitive that adding the cells directly would have purified the plasmid DNA. The only time Burgoyne demonstrates DNA isolation directly from cells is for **isolation of genomic DNA** (Examples 1 and 3). As with Rogers, Burgoyne does not demonstrate **plasmid** DNA isolation on a solid medium or matrix **directly from cells containing a mixture of both genomic DNA and plasmid DNA**.

19. The Rogers paper, the Burgoyne patent, and the Kahn paper, taken either alone or together, neither disclose nor suggest the present invention.

20. The deficiencies of the Rogers paper and the Burgoyne patent are **not** remedied by the disclosure of the Kahn paper. Kahn describes the use of bacterial plasmid as vehicles for the stable maintenance of foreign DNA in bacteria (p. 268). Kahn reviews various plasmid cloning vectors. However, **Kahn teaches away from the present invention, as well as the Rogers paper and the Burgoyne patent**, by emphasizing that the different properties of plasmid DNA and chromosomal genomic DNA can be used to separate these two types of DNA. For example, Kahn states that "[p]lasmid DNA can be separated from chromosomal DNA on the basis of its smaller size or by taking advantage of the unique properties of covalently closed circular DNA molecules" and then describes cesium chloride (CsCl)-ethidium bromide (EtBr) gradient centrifugation as a means "to purify and concentrate the covalently closed plasmid circles" (p. 269; all emphasis added). Additional methods are mentioned, including pH adjustment ("the high pH required for the denaturation of the covalently closed form of DNA"), separation using acid phenol and low salt, and methods using polyethylene glycol (PEG) or hydroxyapatite (p. 271). **These methods underscore the distinctive properties of plasmid DNA vs. chromosomal/genomic DNA and the need for multi-step liquid purification, even for the smaller samples.** As with Rogers and Burgoyne, Kahn does **not** demonstrate **plasmid** DNA isolation on a solid medium or matrix **directly from cells containing a mixture of both genomic DNA and plasmid DNA**.

21. **<u>The teachings of Rogers, Burgoyne, and Kahn, either alone or in combination, do not suggest the present invention.</u>** If anything, taken together, **these references emphasize the need to isolate either <u>genomic DNA without plasmids</u> (Rogers) or <u>previously purified plasmid DNA</u> (i.e., <u>without</u> genomic DNA in the sample) (Burgoyne) and the <u>differences in the properties of the two types of DNA</u> (Kahn).**

22. In contrast, **the present invention specifically mentions the use of plasmids** as the vectors (see, e.g., the Abstract; p. 1, ll. 17-18; p. 6, l. 31; Tables 1, 2 and 3 of Example 1, and Examples 2 and 3) **and the isolation of plasmids directly from a host cell <u>without previous removal/purification from chromosomal/genomic DNA</u>**. The possibility of **directly isolating plasmids from a host cell** or virus in accordance with the present invention was **<u>not</u> obvious** from Rogers, and indeed, Burgoyne, both of which, either alone or together, teach away from the invention by working only with genomic DNA without plasmids or with previously purified DNA (see, e.g., the references to the Burgoyne patent at page 3 (ll. 4-5) and, in particular, from page 14 (l. 18) to page 15 (l. 4), while Kahn only emphasizes the distinctive properties of the two types of DNA. The present invention is directed to plasmids, which are structurally and size-wise different from genomic DNA in that they are much smaller and circular (often supertwisted/supercoiled DNA). While the results of the Rogers paper show that genomic high molecular weight DNA does remain on the FTA® paper following washing, Rogers does not show that such a procedure could result in adequate recovery of plasmid DNA for use in transformation assays. Clearly, Rogers does not use bacterial strains containing these lower molecular weight plasmid structures and as such could not demonstrate that such plasmid DNAs could be recovered as detected by PCR. The present invention shows that plasmid DNA is found in the TE buffer washes (see, e.g., p. 15, ll. 6-22). Thus, it could additionally be argued that the present invention provides a method where the normally discarded wash solution from FTA® contains the biologically active transforming plasmid DNA.

23. The present application demonstrates the ability of **plasmid** DNA to **elute** from the washed punch after a 20-minute incubation in buffer at room temperature such that the **plasmid vector** DNA is **isolated *from* the FTA® medium** (e.g., p. 13, ll. 3-6). In the present application, two different **host *cells*,** bacteria and yeast, each containing plasmid DNA were applied to FTA® cards. The specification shows that the **cells** were lysed and the **plasmid** DNA retained and protected by the FTA® chemicals during room temperature storage (for at least 3 months; p. 18, ll. 28-29). **M13 plaques and cells** infected with **M13 bacteriophage** were also used (see Example 4). As shown in Examples 1-2, ***plasmid* DNA *directly from host cells* can be *eluted by washing* in order to isolate it *from* the FTA® card** (see, e.g., p. 17, ll. 1-17 and Table 4; p. 13, ll. 3-6; p. 14, ll. 16-18 and 25-30).

24. **Plasmid DNA behaves differently from genomic DNA based on its size and its structure. It would not be expected that less complex DNA would interact with a solid matrix in the same manner as genomic DNA, so it would not be intuitive that plasmid DNA could be isolated on a solid matrix..**

25. **Genomic DNA, due to its large size relative to plasmid DNA, behaves differently** under various circumstances. **Differences in the properties of genomic vs. plasmid DNA have been exploited in a wide range of laboratory processes** (e.g., in DNA isolation) in order to separate the two types of DNA. Examples of references demonstrating and/or exploiting the different properties of these two types of DNA include Hansen & Blakesley, "Simple Archiving of Bacterial and Plasmid DNAs for Future Use," Focus 20(3): 72-74 (1998), and Old & Primrose, Principles of Gene Manipulation (4th ed.), Blackwell Scientific Publications (Boston: 1989). For example, it has been shown that plasmid DNA significantly elutes from a punch, as standard fluorescent cycle sequencing of plasmid DNA directly from a washed punch did not detect any signal punch (Hansen & Blakesley, p. 74, c. 1). Clearly Hansen shows that low amounts of plasmid DNA remain on the solid matrix - not when cells comprising plasmids are applied to the matrix, but rather when the plasmids are eluted. Old & Primrose provides examples of the inherently different properties of plasmids vs. genomic. The fact that these protocols are, not surprisingly, different than the

protocols of the present invention does not render them irrelevant. For example, it could not have been assumed that plasmid DNA would elute from bacterial cultures on FTA® medium in the same manner as chromosomal DNA (see, e.g., Old & Primrose, pp. 43-44, as two examples of the different properties of chromosomal DNA and plasmid DNA). While this present invention does not relate either to density in the presence of ethidium bromide, or to denaturation at pH 12.0-12.5, as discussed in Old & Primrose (pp. 43-44), the reference nevertheless underscores the fact that ***differences in the properties*** of chromosomal DNA and plasmid DNA demonstrate the ***lack of predictability*** that a method that works with one type of DNA will work with the other. Instead, these reference have been brought to the attention of the Patent Office as evidence of **the inherently different properties of the two types of DNA** and to show that **the combination of the Rogers, Burgoyne, and Kahn references, which relate to disparate subject matter, would not be obvious.** Moreover, as with Old & Primrose and Hansen, Kahn emphasizes the distinctive properties of the two types of DNA.

26. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 19 March 2009

Walter King
Walter King, Ph.D.